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10/632,794	08/04/2003	Huai-Jen Tsai	8961-000004/US	5554
30596	7590	03/23/2007	EXAMINER	
HARNESS, DICKEY & PIERCE, P.L.C. P.O.BOX 8910 RESTON, VA 20195			BERTOGLIO, VALARIE E	
			ART UNIT	PAPER NUMBER
			1632	
SHORTENED STATUTORY PERIOD OF RESPONSE		MAIL DATE	DELIVERY MODE	
3 MONTHS		03/23/2007	PAPER	

Please find below and/or attached an Office communication concerning this application or proceeding.

If NO period for reply is specified above, the maximum statutory period will apply and will expire 6 MONTHS from the mailing date of this communication.

Office Action Summary	Application No.	Applicant(s)	
	10/632,794	TSAI, HUAI-JEN	
	Examiner Valarie Bertoglio	Art Unit 1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1) Responsive to communication(s) filed on 16 January 2007.
 2a) This action is FINAL. 2b) This action is non-final.
 3) Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4) Claim(s) 1-5, 7, 8 and 10-12 is/are pending in the application.
 4a) Of the above claim(s) _____ is/are withdrawn from consideration.
 5) Claim(s) _____ is/are allowed.
 6) Claim(s) 1-5, 7, 8 and 10-12 is/are rejected.
 7) Claim(s) _____ is/are objected to.
 8) Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9) The specification is objected to by the Examiner.
 10) The drawing(s) filed on 04 August 2003 is/are: a) accepted or b) objected to by the Examiner.
 Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
 Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
 11) The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12) Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
 a) All b) Some * c) None of:
 1. Certified copies of the priority documents have been received.
 2. Certified copies of the priority documents have been received in Application No. _____.
 3. Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) Notice of References Cited (PTO-892)
 2) Notice of Draftsperson's Patent Drawing Review (PTO-948)
 3) Information Disclosure Statement(s), (PTO-1449 or PTO/SB/08)
 Paper No(s)/Mail Date 01/2007.

4) Interview Summary (PTO-413)
 Paper No(s)/Mail Date. _____.
 5) Notice of Informal Patent Application (PTO-152)
 6) Other: _____

DETAILED ACTION

Applicant's reply dated 01/16/2007 has been received. Claims 1-5,7,8, and 10-12 have been amended. Claims 6,9 and 13 have been cancelled. Claims 1-5,7,8 and 10-12 are pending and under consideration in the instant office action.

Specification

The objection to the specification is withdrawn in light of Applicant's removal of the figures from the body of the specification.

The amendment filed 01/06/2007 is objected to under 35 U.S.C. 132(a) because it introduces new matter into the disclosure. 35 U.S.C. 132(a) states that no amendment shall introduce new matter into the disclosure of the invention. The added material which is not supported by the original disclosure is as follows: Applicant has deleted the phrase "or green fluorescent protein from pEGFP-1" at paragraph [0020] and the phrase "or green" at paragraph [0021] of the specification. Applicant fails to explain or set forth that the amendment to the specification at paragraphs [0020] and [0021] are supported by the specification as originally filed. Omission of GFP from the disclosure is considered new matter because it changes what Applicant is conveying as being the invention. This disclosure as originally filed does not support the use of an RFP with the exclusion of GFP.

Applicant is required to cancel the new matter in the reply to this Office Action.

Claim Objections

The previous objections to the claims are withdrawn with the exception of claim 4.

The objection to claims 2 and 4 for containing a drawing is withdrawn in light of Applicant's removal of the drawings from the claims.

The objections to claims 1-3,10 and 12 are withdrawn in light of Applicant's amendments to the claims.

Claim 1 is objected to because it is awkward as written. While it is not unclear what Applicant is intending to claim, the phrase "first inverted terminal repeats (ITR)" is awkward in referring to an ITR from an adeno-associated virus as "first inverted terminal repeats". More concise language would include "a first inverted terminal repeat" rather than use of "first...repeats". The same applies for "second inverted terminal repeats" at lines 4-5. The claim is further awkward in use of the term ""red fluorescence gene product". Preferred language would include "red fluorescent gene product".

Claim 2 is objected to because it is awkward as written. The phrase "first inverted terminal repeats " is awkward in referring an ITR from an adeno-associated virus as "first inverted terminal repeats". More concise language would include "a first inverted terminal repeat" rather than use of "first...repeats". The claim is awkward in use of the term ""red fluorescence gene product". Preferred language would include "red fluorescent gene product". Appropriate correction is required.

Claim 3 is objected to for the following informalities: Applicant has amended claim 3 to limit it to use of a gene encoding a red fluorescent protein (see preamble and Applicant's Remarks at page 16, paragraph 3), however, it appears the limitation was omitted from steps (a) and (b) where it should read that the gene encodes a red fluorescent gene product. Appropriate correction is required.

Claims 10 and 12 are objected to for the following informalities: The body of the claim does not appear to be consistent with the preamble of the claims because the preamble indicates a Markush group wherein only a single group member is listed in the claim. The phrase "selected from the group consisting of" should be deleted.

Double Patenting

Applicant is advised that should claim 10 be found allowable, claim 12 will be objected to under 37 CFR 1.75 as being a substantial duplicate thereof. When two claims in an application are duplicates or else are so close in content that they both cover the same thing, despite a slight difference in wording, it is proper after allowing one claim to object to the other as being a substantial duplicate of the allowed claim. See MPEP § 706.03(k).

Claim Rejections - 35 USC § 112-1st paragraph

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

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The rejection of claims 2 and 4 as failing to comply with the enablement requirement is withdrawn in light of Applicant's amendments to the claims. The claims no longer recite a specific plasmid construct that would require deposit.

The rejection of claim 1 under 35 U.S.C. 112, first paragraph, because the specification, is not enabling for the claimed construct wherein the promoter is not operably linked to the gene encoding a fluorescent gene product is withdrawn in light of Applicant's amendments to the claims requiring operable linkage.

Claim Rejections - 35 USC § 112-2nd paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-5,7,8 and 10-12 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The rejection of claim 1 is withdrawn in light of Applicant's amendment to the claim and Remarks.

Claim 1 remains rejected and claim 10 is rejected for reciting “ α -actin promoter of golden zebrafish”. It is unclear whether the term “promoter” is associated with the “ α -actin gene” or with the “golden zebrafish”. Claim 10 was previously rejected for reciting “promoter – for golden zebrafish”. Applicant has amended the claim to recite “promoter of golden zebrafish”,

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which is unclear as set forth above. Claim 2 depends from claim 1. Claim 11 depends from claim 10.

The following grounds of rejection are necessitated by Applicant's amendment to the claims.

Claims 2,4 and 8 are unclear because they recite that the transgene is operably linked to the pUC backbone. This appears to be an incorrect representation of the invention as transgenes are not usually operably linked to the vector backbone carrying them. Clarification is not found in the specification. Thus, it is not clear what is operable linkage between the backbone and the transgene.

Claim 7 is unclear because it is not clear the metes and bounds of the term "systemic" are not clear and are not defined by the specification. Thus it is not clear if the claim is requiring expression in all muscle tissues of the fish, or homogeneously in one or more specific muscle tissues. It is noted that the term "systemic" is used in claim 3, however, the claim is clearly limited to a fish having red fluorescence in the skeletal muscle, which broadly, but clearly, encompasses any degree of or variation of expression pattern in skeletal muscle. Claim 8 depends from claim 7.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

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(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

The rejection of claims 1 and 2 under 35 U.S.C. 102(b) as being anticipated by Chou *et al.* [Transgenic Research, 10:303-315, August 2001, IDS] or Hsiao et al [Developmental Dynamics, 220:323-336, April 2001] as evidenced by <http://www.rzpd.de/info/vectors/pCS2plus.shtml> (printout attached).is withdrawn in light of Applicant's amendments to the claims. The claims are now limited to use of a red fluorescent protein, which was taught by neither Chou nor Hsiao. However, the claims are added to the rejection under 35 USC 103, below, as necessitated by the above-mentioned amendment.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

1) The rejection of claims 1-4 and 6,7 and 9-11 under 35 U.S.C. 103(a) as being unpatentable over Hsiao et al. [Developmental Dynamics, 220:323-336, April 2001] in view of Carvan et al [Ann. N.Y. Acad. Sci. 919:133-147, 2000] is withdrawn in light of Applicant's amendments to the claims limiting them to use of DsRed or a gene encoding red fluorescence. Hsiao and Carvan taught use of GFP.

2) Claims 5,8 and 12 remain rejected and claims 3-4,6,7 and 9-11 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hsiao et al. [2001] in view of Carvan et al [2000] and

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further in view of Finley et al, [Biotechniques, 31:66-72, July 2001]. The rejection is maintained for reasons of record set forth at pages 11-12 of the office action dated 03/03/2006 and reiterated below. The rejection is applied to claims 3-4,6,7 and 9-11 which were amended to limit claims to a gene encoding a red fluorescent protein, specifically DsRed.

Hsiao taught a method of making a transgenic zebrafish with systemic fluorescence of the skeletal musculature by injecting a linearized plasmid including flanking ITRs, an α -actin promoter that replaced a CMV promoter, a fluorescent EGFP gene and an SV40 polyA, into fertilized zebrafish embryos and allowing fluorescent embryos to develop into zebrafish (Figure 1, second construct; page 325, col. 2, paragraph 2; page 333, col. 2, paragraphs 1 and 3). Hsiao taught using leopard strain zebrafish because they have less pigmentation than the AB strain (page 333, col. 2, paragraph 4). Lack of pigmentation is important in visualizing fluorescence (see page 323, col. 2, lines 1-2). The limitations of the claims requiring presence of a pUC backbone, which comprises a NotI site, are met by Hsiao as set forth at pages 10-11 of the office action dated 07/13/2006.

Hsiao et al did not teach using the golden strain of zebrafish as claimed.

However, the golden mutant strain of zebrafish was well known at the time of filing to produce less pigment as a result of a mutation at the *golden* locus. Carvan et al. taught the use of *golden* mutants to make transgenic zebrafish comprising transgenes encoding fluorescent gene products (page 141, paragraph 5). Carvan taught that golden mutants are preferred over other pigmentation mutants, such as *albino*, because *albino* mutants are poor breeders. Neither Carvan nor Hsiao et al. taught use of a gene encoding a red fluorescent protein.

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However, Finley et al. taught the use of several different fluorescent reporters in zebrafish, including DsRed. Finley also taught properties unique to DsRed such as low turnover and a unique emission spectra. Furthermore, Finley et al. taught that DsRed has a high signal to noise ratio, optimizing it as a reporter gene.

It would have been obvious to one of ordinary skill in the art at the time of filing to combine the technology taught by Hsiao of using ITR elements to enhance fluorescent reporter gene expression in transgenic zebrafish with the teachings of Carvan et al. in using golden mutant zebrafish and Finley teaching use of DsRed as a fluorescent reporter. One of skill in the art would have been motivated to combine these teachings of Hsiao et al. with those of Carvan et al. to make the fluorescent transgene product more readily visible as visualization would not be obscured by pigment that arises in wild-type zebrafish beginning at day 3 of development. One of skill in the art would have been motivated to combine these teachings of Hsiao et al. and Carvan et al. with those of Finley et al. because Finley et al taught advantages of DsRed over GFP as well as uses for multiple fluorescent reporter genes in the same fish.

One of skill in the art would have a reasonable expectation of success in combining the teachings of Hsiao et al. with those of Carvan et al. because the *golden* mutants are of the same species as the *leopard* mutants and differ only at the respective pigmentation loci. One of skill in the art would have a reasonable expectation of success in additionally combining the teachings of Finley et al. because the molecular techniques to make the claimed DsRed transgene were known and Finley taught transgene stability, expression and visualization.

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Applicant's arguments have been fully considered and are not found persuasive.

Applicant argues that it is clear from the art (Rahman reference, submitted with Remarks dated 01/16/2007) that it is unpredictable whether a particular gene will be expressed, even when the genes are driven by the same promoter (page 16 of Applicant's Remarks).

In response, this argument is not persuasive because Applicant points to teachings of Rahman regarding results using lacZ transgenes in mice, not genes encoding fluorescent proteins in fish. In fact, Applicant points to teachings of Rahman that indicate that lacZ has an effect on promoter activity that is not observed with other reporter genes (see page 418, col. 1, paragraph 3). Furthermore, it appears Rahman is teaching variability between lines of transgenic medaka fish, based on the site of transgene insertion and copy number, not based on the effect of the specific reporter gene on promoter activity. Thus, it appears Applicant is trying to apply the teachings of Rahman in setting forth that use of dsRed or another gene encoding a red fluorescent protein may have an effect on the activity of the promoter. However, a basis for this extrapolation of teachings of Rahman concerning the effect of lacZ on promoter activity to DsRed and other genes encoding red fluorescent proteins is not found.

Applicant argues that the Tsai Declaration submitted 01/16/2007 establishes that replacement of EGFP with RFP in zebrafish is not easily derived by one of skill in the art (page 17, paragraph 1; page 19, paragraph 2 of Applicant's Remarks). Applicant argues that given the uncertainty of gene expression, one of skill in the art would not have a reasonable expectation of success that such a combination of teachings such that one could obtain adult fish expressing red systemic fluorescence.

In response, the uncertainty of gene expression is addressed above. Any established uncertainty of gene expression in substituting a RFP gene in place of a GFP gene is merely in copy number and insertion position, which can be remedied by making multiple transgenic lines, and does not constitute undue experimentation to obtain that which is claimed. With respect to the Tsai declaration, the Tsai declaration under 37 CFR 1.132 filed 01/16/2007 is insufficient to overcome the rejection of the claims based upon obviousness under 35 USC 103(a) as set forth in the last Office action. It appears Applicant may be attempting to argue that unexpected results were obtained. The Tsai declaration sets forth that it took many years to develop a red fluoresce that results in the claimed transgenic fish and that the WAUED is ideal. This statement is not effective in overcoming the instant rejection as it is not known what WAUED is, nor is the claimed invention limited to such. In fact, if the only red fluorescent protein that is effective is “WAUED”, then it would appear that the claims would not be enabled for greater breadth beyond the WAUED red fluorescent protein referred to be the Tsai declaration. Furthermore, support relevant to “WAUED” is not found in the specification.

Applicant disputes the contention that visible systemic fluorescence in adult fish is an inherent feature of transgenic fish that incorporate a gene encoding a fluorescent gene product. Applicant argues that two strains will not necessarily express fluorescence in an identical or even similar manner. Applicant refers to the teachings of Opsahl *et al.* (page 17, paragraph 2 of Applicant’s Remarks). Opsahl *et al.* teach that transgene expression can be altered by the presence of strain specific modifiers.

In response, the teachings of Opsahl *et al* are not persuasive. Firstly, Opsahl *et al* is relevant to mouse species and not to fish strains. Secondly, golden and leopard zebrafish strains differ somewhat in genetic background due to inbreeding of the line and maintenance of the corresponding pigment gene mutation. Such genetic differences are generally known in the zebrafish art to have an impact on the expressivity of some mutations and could potentially affect the level of promoter activity or transgene expression based on position effect, however, the effects on promoter activity would not be commonplace and predicted to occur with most promoters and position effect variegation is easily overcome by generating additional lines of transgenic fish. Furthermore, it is noted that the instant claims are not limited to any specific strain and the specification is not so limited either, and thus there is no evidence on the record that the teachings of Opsahl *et al.* are relevant to the instantly claimed invention.

Applicant maintains that analysis of expression of a gene on a tissue-to-tissue basis demonstrated that gene expression in stably transformed fish occurred with variable intensity in different organs and tissues as noted in Rahman *et al*. Applicant reiterates that the expression of a transgene can be influenced by the promoter driving the transgene, the copy number and the position of integration (page 19 of Applicant's Remarks). Thus, Applicant asserts that expression of the claimed transgene would be unpredictable.

In response, the claimed α -actin promoter is demonstrated in the art to be active in zebrafish with no indications that there are any inherent characteristics of this promoter that make it sensitive to genetic background, position effect or copy number to any degree that cannot be overcome by making multiple lines of fish. If there were such unpredictability in the instant

case, then it would be held that success in making transgenic fish is entirely unpredictable, which is clearly not the case.

Applicant argues that prior works has shown that fluorescence may be expressed in transgenic fish in embryos and larval stages and can be lacking in adult fish (page 19, paragraph 1 of Applicant's Remarks). Applicant contends that because the scales of the fish cover the skeletal muscle, the fluorescence will be obscured. Applicant argues that the Examiner's suggestion that fluorescence will be observable in adult fish is mere speculation. In response, expression in adult stages is a property of the promoter used. That in the applied art and the instant invention is the same. The art provided suggests use of non-pigmented fish to increase visibility of fluorescence. Applicant has not provided any evidence that such fluorescence would not be visible. In fact, Figure 7 depicts an adult fish visibly expressing GFP.

Applicant reiterates that the claims have been amended to recite use of a red fluorescent gene and that the fish have been limited to adult fish (page 20, paragraph 1). These amendments fail to overcome the instant rejection for reasons set forth above.

Applicant argues that Finley did not teach fluoresce of DsRed driven by the *Xenopus* EF1a promoter and that expression from the EF1a promoter is patchy in embryos rather than in a "systemic" pattern of the instant invention.

In response, Finley is not relied upon for the pattern of gene expression. Rather, such limitations are taught by Hsiao *et al*, which uses the same promoter as the instant invention.

Finley is merely used to provide relevant teachings and motivation to use a different gene encoding a fluorescent gene products in place of the GFP gene taught by Hsiao *et al.* Applicant states in Table 1 of the Remarks at page 20 and par a1 of page 21, that Hsiao et al. use the β -actin promoter. Applicant is directed to Figure 1, second construct, of Hsiao *et al.*, which demonstrates that the α -actin promoter was taught and used by Hsiao, rendering Applicant's arguments moot.

The following new rejection is necessitated by amendment to claims 1 and 2 limiting them to a gene encoding a red fluorescent protein.

3) Claims 1 and 2 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hsiao et al. [2001] in view of Finley et al, [Biotechniques, 31:66-72, July 2001].

Hsiao taught a method of making a transgenic zebrafish with systemic fluorescence of the skeletal musculature by injecting a linearized plasmid including flanking ITRs, an α -actin promoter that replaced a CMV promoter, a fluorescent EGFP gene and an SV40 polyA, into fertilized zebrafish embryos (Figure 1, second construct; page 325, col. 2, paragraph 2; page 333, col. 2, paragraphs 1 and 3). The limitations of the claims requiring presence of a pUC backbone are met by Hsiao as set forth at pages 10-11 of the office action dated 07/13/2006. Hsiao *et al.* did not teach use of a gene encoding a red fluorescent protein.

However, Finley et al. taught the use of several different fluorescent reporters in zebrafish, including DsRed. Finley also taught properties unique to DsRed such as low turnover

and a unique emission spectra. Furthermore, Finley et al. taught that DsRed has a high signal to noise ratio, optimizing it as a reporter gene.

It would have been obvious to one of ordinary skill in the art at the time of filing to combine the technology taught by Hsiao of using ITR elements to enhance fluorescent reporter gene expression in transgenic zebrafish with the teachings of Finley regarding use of DsRed as a fluorescent reporter. One of skill in the art would have been motivated to combine these teachings of Hsiao et al. with those of Finley et al. because Finley et al taught advantages of DsRed over GFP as well as uses for multiple fluorescent reporter genes in the same fish.

One of skill in the art would have a reasonable expectation of success in combining the teachings of Hsiao et al. with those of Finley et al. because the molecular techniques to make the claimed DsRed transgene were known and Finley taught transgene stability, expression and visualization.

Thus, Applicants' claimed invention as a whole is *prima facie* obvious in the absence of evidence to the contrary.

Many of Applicant's arguments to the preceding rejection of claims 3-12 under 35 USC 103(a) are relevant to the instant rejection. These arguments are addressed above and applicable to the instant rejection.

Conclusion

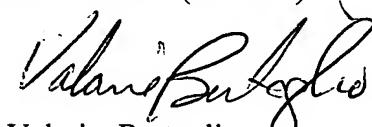
Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Valarie Bertoglio whose telephone number is (571) 272-0725. The examiner can normally be reached on Mon-Thurs 5:30-4:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Peter Paras can be reached on (571) 272-4517. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).



Valarie Bertoglio
Examiner
Art Unit 1632